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The calcium content of the body tissues and castings of the earthworm *Pontoscolex corethrurus* (Annelida, Oligochaeta)

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With 3 figures

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1. Introduction

In recent years the role of earthworms in incorporating dung and vegetable organic matter into soil and increasing available N and essential minerals has been emphasized (LUNT & JACOBSON (1944), SHRIKHANDE & PATHAK (1948), NIJHAWAN & KANWAR (1952), NYE (1955), SATCHELL (1958, 1963), ABATUROV (1972). According to PIEARCE (1972), species with active calciferous glands absorb excess Ca from their diet and transfer it to calciferous glands from which it is excreted via the digestive tract. Calciferous glands may also have a role in maintaining the acid base balance in the system.

Pontoscolex corethrurus (FR. MÜLL.) is a common earthworm inhabiting gardens around Bangalore; it has well developed calciferous glands. In the present study an attempt has been made to investigate the partitioning of Ca between earthworm body tissues, castings and soil.

2. Materials and methods

2.1. Introductory note

P. corethrurus is found in its highest numbers in mango *(Mangifera indica)* gardens (population density in four such gardens was 3,250–6,790 Ind./m^3 [1,150–2,400/100 cft.] after rains). In the laboratory 400 to 500 worms were introduced into pots filled with garden soil which contained 1 kg weathered and dried mango leaf powder dispersed in one tonne of most soil. The mango leaves contained 30.35 mg% Ca and 40 : 0.8 ratio of carbon to nitrogen. From these pots worms were taken out and left in Petri dishes on wet filter paper for 3 to 7 days until the mud was cleared from their guts before they were used for Ca assay.

2.2. Ca in whole animals

Worms were weighed, dried in an hot air oven (110 °C) overnight, and reweighed. The dried material was then wet ashed for total Ca estimation: 10 mg was treated with 1 ml H_2SO_4 and digested at 120 °C for 3 hr in an autoclave. The digest was neutralized with 18 N NaOH, and distinctively made alkaline with the ammonium hydroxide solution. Then 1 ml of 4% ammonium oxalate was added to precipitate the Ca. After centrifugation at 100 rev. min^{-1} for 15 min the precipitate was washed three times with methanol to remove pigments and collected after recentrifugation, for Ca-assay. The Ca-oxalate precipitate was dissolved in 2 ml 1 N H_2SO_4 and Ca estimated by compleximetric method (OSER 1965).

2.3. Protein bound calcium

A 5% homogenate of the worm in 0.25 M sucrose was prepared. Half of the homogenate was saturated with lanthanum chloride in 15 ml centrifuge tubes. The precipitate was collected after centrifugation at 5,000 rev. min^{-1} for 15 min at 4 °C. The process was repeated once more and the precipitate wet ashed and its Ca content estimated as described in the preceding section. Protein in the other portion of the sucrose homogenate was precipitated with LaCl and its N content was estimated by the Micro-Kjeldahl method (OSER 1965).

2.4. Calcium binding capacity

Worm tissue (50 mg) was homogenized in 3 ml 0.1 M calcium acetate. Then a 10 cm length of wet cellophane tubing was tied at one end to form a sac, The homogenate was inserted into the cellophane tube, and the top tied off above the solution. A section of tubing was allowed to collapse above the solution next to the knot to provide for expansion of the solution and to prevent stretching of the membrane. Dialysis was carried out in 20 ml 0.1 M calcium acetate at 25 °C for 24 hr agitating the medium continuously with a magnetic stirrer. The Ca content of the dialysate was estimated by compleximetric method (Oser 1965). The contents of the dialysis tube were collected after the experiment and their protein content estimated (Micro-Kjeldahl method).

2.5. Calcium in the body tissues

Five mg samples of calciferous glands, digestive tracts and body walls of the worms were excised and homogenized in 5 ml distilled water. Each homogenate was centrifuged at 2,000 rev. min^{-1} for 20 min and the supernatant decanted for Ca assay. The residue was then rehomogenized in 5 ml 10% acetic acid and centrifuged to collect the supernatant. The remaining residue was rehomogenized in 5 ml 1% HCl, centrifuged, and the supernatant collected. The Ca contents of these extracts were estimated by a compleximetric method (Oser 1961).

2.6. Calcium in the castings and soil organic matter (leaf litter)

Twenty five worms were left on a previously weighed filter paper in a (15 cm diameter) Petri dish for 24 hr. The dish was humidified with a few drops of water. The filter paper and excrements on it were then dried in an oven for overnight at 110 °C and weighed. The whole paper with dried castings was then homogenized in 5 ml water, acetic acid and HCl as described in the earlier paragraph and soluble, exchangeable and insoluble Ca were estimated in the supernatants by a compleximetric method (Oser 1965). Oven dried 10% (Wt/Vol.) soil homogenate was treated as above for soluble, exchangeable and insoluble Ca assay.

2.7. Soluble carbonate in castings soil and organic matter

Samples of castings and soil were each weighed and 10% aqueous homogenates were prepared. Supernatants were collected after centrifugation, and the residue then dried in an oven at 110 °C. The supernatants containing the soluble carbonates and the residues retained insoluble carbonates. The residue or supernatant solution was mixed with dil. HCl in a Warburg flask, and the CO_2 evolved measured manometrically (Precision Instruments Co., Chicago). Fifty mg samples of residue in 2 ml water, or 2 ml samples of supernatant, were taken in the central well and 1 ml 25% HCl was tapped from the side arm after equilibrating the manometers at 25 °C. Readings were corrected for changes in temperature and pressure. CO_2 evolved from soluble and insoluble carbonates was calculated per gram and the ratios between CO_2 evolved from soluble and insoluble carbonates n soil and castings compared.

2.8. *p*H of the gut

The buccal, gizzard, and oesophageal regions, anterior intestine and posterior intestine of the gut were excised. The tissues were separately homogenized in distilled water and *p*H of the homogenates were measured with a *p*H meter (Elico, Hyderabad).

2.9. Free and total acids in the gizzard

Gizzards of 15 worms were excised and transferred into a cavity block. These were opened and rinsed with 0.5 ml distilled water. The washing was treated first with Topfer's reagent (Oser 1965) to test for free acidity and titrated against 0.1 N NaOH for total acidity with a drop of phenolphthalein.

3. Results

The total Ca and the protein bound Ca of the worms decreased with the increase in weight of the body (Figs. 1 and 2). The Ca binding capacity of the proteins was higher in worms of a lower weight range than in heavier worms (Fig. 3).

The Ça content of the calciferous glands was proportionately lower in larger worms (Table 1). Previous experiments in the laboratory have shown that wet weight of the worm increases proportionately with age. Ca was found to occur in free (water soluble), exchangeable (acetic acid soluble) and insoluble (HCl soluble) forms. In the larger-sized individuals more than 25% of total body Ca accumulated either as free, acetic acid soluble or insoluble Ca in the calciferous glands. The ratio of the available forms of Ca varied among tissues (Table 2). Calciferous glands contained more ionic Ca than exchangeable or insoluable Ca.

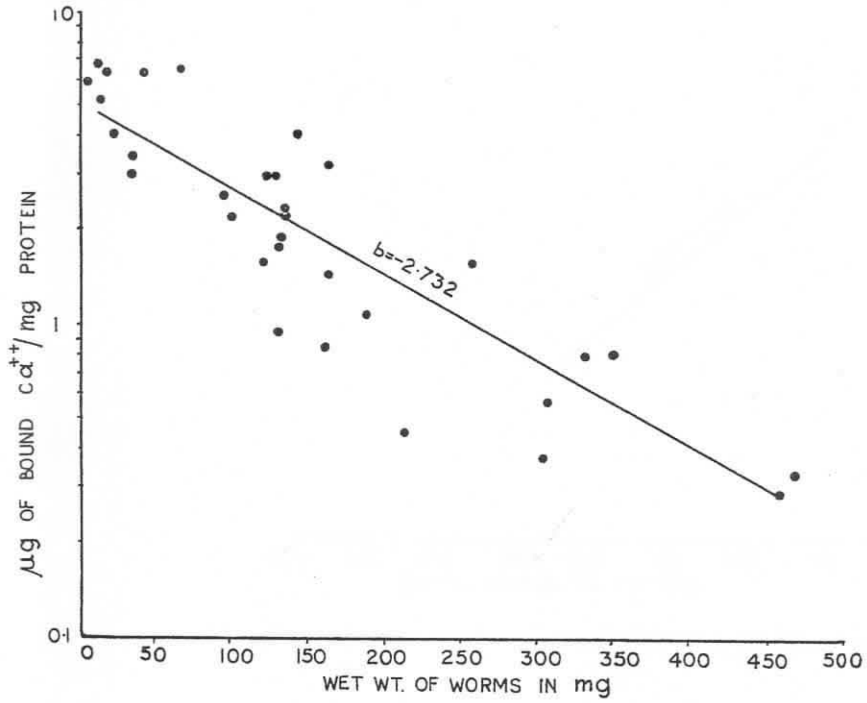

Fig. 1. *Pontoscolex corethrurus:* Influence of worm size on the total calcium content of the worm.

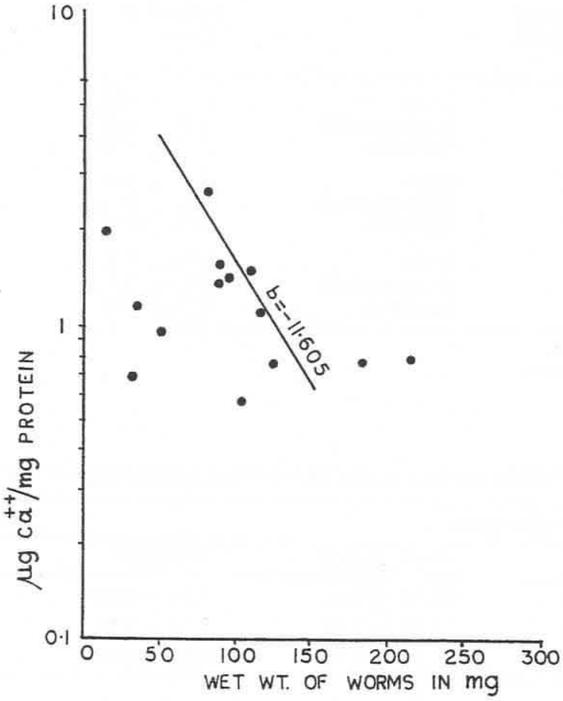

Fig. 2. *Pontoscolex corethrurus:* Influence of worm size on the protein bound calcium contents.

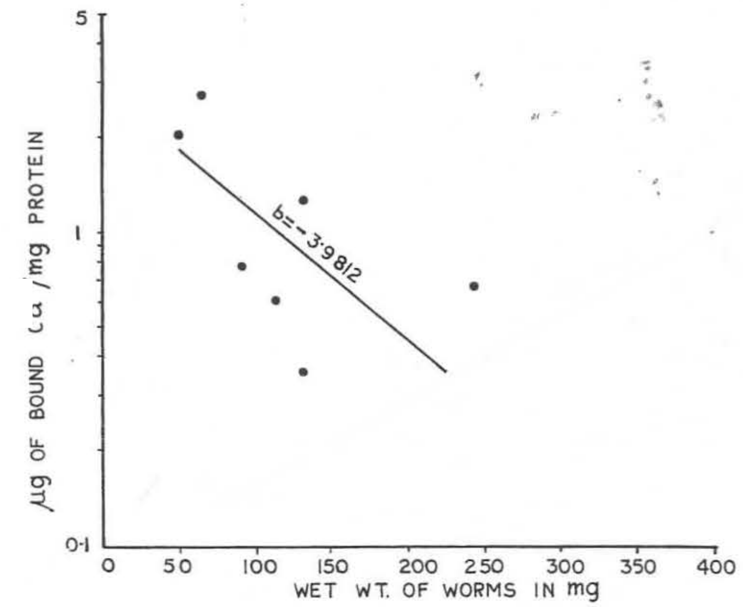

Fig. 3. *Pontoscolex corethrurus:* Influence of worm size on the calcium binding capacity of the total proteins.

Table 1. *Pontoscolex corethrurus:* Calcium content of calciferous glands

Wet weight of worm (mg)	Mean gland wet weight (mg)		μg Ca mg^{-1} gland
5 – 50	0.06	Ionic	1.42 ± 0.31
		Exchangeable	0.47 ± 0.10
		Insoluble	0.35 ± 0.08
80 – 200	0.27	Ionic	2.89 ± 0.04
		Exchangeable	1.94 ± 0.04
		Insoluble	0.62 ± 0.03
200 – 400	1.60	Ionic	3.00 ± 0.81
		Exchangeable	2.05 ± 0.13
		Insoluble	0.22 ± 0.01

Values are mean ± S.D. of 3 sets of samples.

Table 2. *Pontoscolex corethrurus:* Tissue calcium content in the 200 – 400 mg weight range of worms

Tissue	μg Ca/g wt. of the worm		
	Ionic	Exchangeable	Insoluble
Calciferous gland	15.61 ± 3.17	10.35 ± 2.41	2.48 ± 0.12
Digestive tract	12.31 ± 2.74	6.76 ± 1.75	5.37 ± 0.55
Body wall	10.64 ± 1.87	6.06 ± 0.34	7.89 ± 2.02

Values are mean ± S.D. of 5 observations.

Table 3. *Pontoscolex corethrurus:* Calcium and carbonates in soil and castings

Constituent	µg/g dry weight	
	Soil	Castings
Ionic calcium	12.24 ± 0.41	145.0 ± 9.81
Exchangeable calcium	12.83 ± 0.37	95.23 ± 7.28
Insoluble calcium	179.62 ± 0.02	32.09 ± 0.93
Ionic/Insoluble carbonate	0.15 ± 0.01	6.98 ± 2.22

Values are mean ± S.D. of 6 observations,

All three tissues contained the highest concentration of Ca as free (ionic) calcium (Table 2). Non-ionic (insoluble) Ca was more in the body wall than in the digestive tract or calciferous glands (Table 2).

The Ca content of the castings varied both quantitatively and qualitatively in comparison to soil. The concentration of soluble (ionic) calcium of castings was 11.8 times more than in surrounding soil but the total calcium was only 1.3 times more in castings (Table 3). Soluble carbonate was more abundant in castings than in the surrounding soil (Table 3).

The *p*H of the buccal region was slightly basic (7.2), that of the gizzard region was slightly acidic (6.8) and that of the oesophagus, anterior and middle intestine regions was alkaline and varied between 8.4 and 9.3. The *p*H of the posterior region of the intestine was neutral (7.0).

The gizzard showed the presence of considerable free acidity; total acidity was 0.13 ± 0.08 mEq.

4. Discussion

The role of the calciferous glands of earthworms is not clear. Robertson (1936) suggested that they secrete amorphous $CaCO_3$ coated with mucus, but he did not mention that the secretions would affect the *p*H of the intestinal fluid. He presented evidence for the possibility that alkaline secretions from the wall of the intestine were responsible for the decreased acidity in that part of the gut. Jeuniaux (1969) maintained that they functioned to excrete excess $CaCO_3$ absorbed from the soil. In contrast to these views, Nijhawan & Kanwar (1952) and Piearce (1972) looked at the casts of earthworms and showed that the castings provide free Ca to soil. The results of the present study suggest that the majority of the Ca eliminated from the body in the casts is in a soluble form. The castings of *P. corethrurus* are rich in water soluble Ca compounds, whereas the soils inhabited by these worms are rich in $CaCO_3$. If one can equate free Ca with extractable Ca, contrary to Robertson (1936) it could be suggested that it is the material secreted by the calciferous glands that raises the *p*H of the material in the alimentary canal. Defaecation of castings thus increases both soluble carbonates and soluble calcium. Piearce (1972) presents the data for acetic soluble faecal Ca.

Satchell (1958) has reported that worm activity in soils increases the base exchange acpacity of the soils. The soluble carbonate in the excrement will add to the exchangeable base content of the soil.

The position of the calciferous glands in the *Pontoscolex corethrurus*, in segments 7—9, arising dorsally from the oesophagus, would seem to mitigate against their acting as a source of Ca secreted into the buccal cavity. Most of the secretion passing into the oesophagus would be carried presumably posteriorly by material passing back along the alimentary canal. In the intestine, the esterification to Ca humate may take place from decaying organic matter (Swaby 1949). Apart from this, a part of the ionic Ca may be excreted and a part absorbed into circulating fluids to enter into metabolic reactions at the tissue level. There is no published evidence that the calciferous glands secrete Ca as an organic salt. Robertson's (1936) work suggests that neutralization of the gut contents is not the major function of the glands. The accumulation of $CaCO_3$ in the oesophageal pouches of some species, e.g. *Lumbricus terrestris*, in the form of large concretions, may well have evolved as a means of ensuring that the $CaCO_3$ secreted by the glands is not broken down and the Ca is not brought into solution further along the gut.

The results on total Ca content of worms are interesting. The total calcium increases in mammalian tissues with age (Weller 1956, Tayler et al. 1962). Our findings indicate, however, that Ca content decreased with increase in weight of the worm. Since the protein bound Ca and Ca binding capacity of proteins of the worm also decreases as size increases, the chelation of Ca^{++} to the proteins may thus be reduced as the worm grows. Consequently, the worm expels most of the assimilated Ca through castings.

From the data presented in Table 3 it is possible to prepare a Ca budget as follows: The difference between the insoluble Ca content of the casts and that of the soil and organic matter is presumably equivalent to the amount of Ca absorbed in the gut of the worm and subsequently secreted by the calciferous glands. This amounts to approximately $130\,\mu g$ dry wt. day^{-1}. Assuming that a worm weighing 150 mg passes something of the order of 5 mg dry weight of soil and organic matter through its gut in a day then would require an output of $0.65\,\mu g$ Ca day^{-1} from the glands. The turnout of gland Ca is about $1.3\,\mu g$ for a worm of this size, from the data in Table 1, twice the estimated daily output.

A mean residence time of 2 days for calciferous gland Ca is quite consistent with published data (Piearce 1972). However, there is an important point to take into account, the possibility that these animals are selective in the material that they consume and that as a result values of Ca in the casts are not directly comparble with those in the soil. It is also evident from Table 3, that the total Ca content of the casts is considerably higher than that of the soil because the animals are feeding on Ca rich materials, e.g. leaf fragments, preferentially.

5. Summary

Earthworms [*Pontoscolex corethrurus* (Fr. Müll.)] were cultured in earthen pots containing soil rich in leaf litter to study their role in conversion of insoluble Ca compounds in the soil into soluble Ca.

The total Ca, protein bound Ca and Ca binding capacity of worms of different weights were assayed; the Ca concentration in worms decreased with increase in worm weight.

Less Ca (as a proportion of worm weight) was stored in the calciferous glands of heavier worms. The glands were poorly developed in worms weighing less than 200 mg.

The castings of this earthworm were rich in soluble forms of Ca and in soluble carbonates. Soluble carbonates will contribute to the exchangeable base content of the castings rather than the base exchange capacity.

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